

## Assessment of metal contamination in the upper reaches of the Tichá Orlice River

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**ABSTRACT:** The aim of the present study was to assess metal contamination in the same reaches of the river, and thus to help explain unsatisfactory reproduction results in the reproduction of salmonoid fish. The contamination assessment was based on measuring metal concentrations in the brown trout (*Salmo trutta morpha fario*) and some bottom sediment samples. The samples were collected in June 2000 and 2001 at two collection sites from the Tichá Orlice River (Červená Voda – 103rd river km; Lichkov – 93rd river km) and its tributary Kralický Brook (100th river km). At each of the sites, 14 brown trouts were collected in each of the periods of monitoring. The AAS method was used to determine the total mercury, arsenic, cadmium, lead, copper, zinc, chromium and nickel contents in the muscle tissue of the fish and in aquatic sediments. The Kralický Brook is an important anthropogenic source of metal contamination (particularly of mercury and copper) for the Tichá Orlice River. The highest concentrations of mercury in muscles of brown trout were found at the Kralický Brook ( $0.37 \pm 0.08$  and  $0.40 \pm 0.08$  mg/kg) and Lichkov ( $0.41 \pm 0.10$  and  $0.34 \pm 0.07$  mg/kg) in 2000 and 2001 and the lowest concentration at Červená Voda ( $0.017 \pm 0.02$  mg/kg in the same years). Significantly higher concentrations of arsenic (in 2000:  $0.30 \pm 0.08$  mg/kg; in 2001:  $0.38 \pm 0.07$  mg/kg) were found in the muscle tissue of the brown trout collected at Červená Voda than at the downstream site Lichkov (in 2000:  $0.18 \pm 0.09$  mg/kg; in 2001:  $0.14 \pm 0.07$  mg/kg). The authors hypothesize that the difference was due to different conditions (principally water temperature). It seems reasonable to assume that unsatisfactory results in the reproduction of fish from the upper reaches of the Tichá Orlice River are due not only to organic pollutants but also to mercury compounds that are classified among suspect endocrine disruptors.

**Keywords:** Elbe tributary; *Salmo trutta morpha fario*; brown trout; muscle; mercury; arsenic; AAS

The upper reaches of the Tichá Orlice River and Kralický Brook came under scrutiny of ichthyopathologists and ichthyotoxicologists in the late 20th century. At various sites of the headwaters of the river, adult brown trout (*Salmo trutta morpha fario*) and grayling (*Thymallus thymallus*) were caught and used for artificial reproduction. The results of the reproduction, however, were not satisfactory. The stretch of the Tichá Orlice between the 59th and the 100th river km is rightly considered a contaminated area (Korunová *et al.*, 1997). Until 1990, the Tesla Company operated a manufacturing facility in the town of Králíky where mercury was used

in the production of fluorescent lights. Lusková *et al.* (1997) identified Tesla Králíky at the Kralický Brook as the main source of mercury contamination of the brown trout. Besides mercury, organic pollutants were also monitored at the Tichá Orlice River (Kredl *et al.*, 1989; Svobodová *et al.*, 1999). The results obtained by Svobodová *et al.* (1999) showed that Kralický Brook discharged significant amounts of organic pollutants (PCB, PAH, OCS, HCB, DDT and its metabolites, HCH) to the Tichá Orlice.

The principal indicator of metal contamination in the upper reaches of rivers is fish tissue (Svobodová *et al.*, 1996). In the study area of the Tichá Orlice

River and its tributary, the Kralický Brook, the principal indicator species is the brown trout. It is a dominant species there from the aspect of both the fish biota and the local angling activities.

The present paper gives the results of a study of metal content in the muscle tissue of brown trout collected at three sites along a 10 km stretch of the Tichá Orlice River and its tributary. The aims of the study were:

- to measure contamination levels at individual collection sites and thus to help explain the reasons for unsatisfactory results in artificial reproduction; and
- to compare metal contents in muscle tissue of brown trout with public health standards, and thus to help implement the food safety strategies.

The study is a follow-up of research into organic pollutant contamination of the upper reaches of the Tichá Orlice River and their role in endocrine disruption in the brown trout population (Kolářová *et al.*, 2004).

## MATERIAL AND METHODS

The fish (*Salmo trutta morpha fario*) were collected at sites along the upper reaches of the Tichá Orlice

River (the right-hand side tributary of the River Elbe) in June 2000 and June 2001. The collection sites were Červená Voda at the Tichá Orlice (103rd river km), the tributary of the Tichá Orlice – Kralický Brook (100th r. km) and Lichkov at the Tichá Orlice (93rd r. km) (Figure 1). The fish were collected after they were stunned using an electric generator set. At each site, 14 brown trouts were thus collected (7 females and 7 males). The caught fish were immediately weighed, their age was determined from their scales, and tissue samples for metal content assessment were collected. The sex of the fish was determined macroscopically and checked by the histological examination of gonads. The main characteristics of the fish species examined in this study are summarised in Table 1. Muscle tissue samples were placed in polyethylene bags, labelled and stored in a freezer at  $-18^{\circ}\text{C}$ .

Muscle samples of individual brown trout were analysed for the content of mercury, arsenic, lead, cadmium, copper, zinc, chrome and nickel. The total mercury content in fish muscle tissues was determined by the AAS method using a single-purpose mercury analyser AMA-254 (ALTEC Ltd.). The determination of Pb, Cd, Cr, Cu and Ni in fish muscle tissues was performed by the ETAAS, and the Zn level was determined by the FAAS (Z-5000

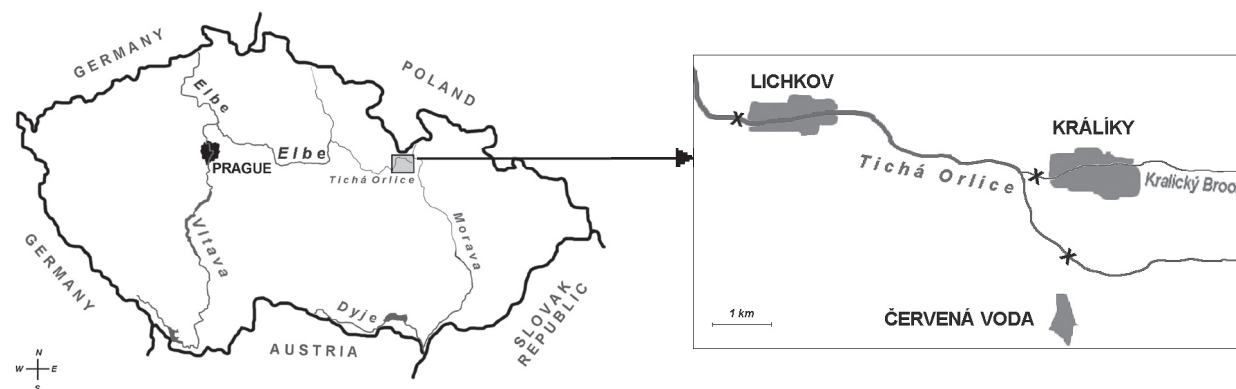


Figure 1. Map of the Czech Republic and location of sampling sites in the present study

Table 1. The main characteristics of the brown trout population sampled

Collection site	2000			2001		
	Fish weight (g)	Age (years)		Fish weight (g)	Age (years)	
	mean $\pm$ SD (n)	min – max		mean $\pm$ SD (n)	min – max	
Červená Voda	81 $\pm$ 25 (14)	2 <sup>+</sup> – 3 <sup>+</sup>		107 $\pm$ 34 (14)	2 <sup>+</sup> – 3 <sup>+</sup>	
Králíky	142 $\pm$ 26 (14)	2 <sup>+</sup> – 3 <sup>+</sup>		137 $\pm$ 26 (14)	2 <sup>+</sup> – 4 <sup>+</sup>	
Lichkov	127 $\pm$ 35 (14)	2 <sup>+</sup> – 3 <sup>+</sup>		139 $\pm$ 19 (14)	2 <sup>+</sup> – 4 <sup>+</sup>	

n = number of fishes examined

apparatus from Perkin Elmer was used in all tests) after mineralization. Mineralization of fish muscle tissue for the determination of metal contents was carried out in microwave-heated laboratory autoclaves, using nitric acid and hydrogen peroxide (Uniclever, Plasmatronica). Samples for the determination of arsenic were processed as above and ashed in a muffle oven (450°C) with an addition of magnesium nitrate. The ash was dissolved in hydrochloric acid,  $\text{As}^{\text{V}}$  was reduced to  $\text{As}^{\text{III}}$ . Arsenic levels were determined by the hydride technique on the MHS-20 apparatus attached to an atomic absorption spectrometer. The accuracy of the results was validated by the following standard reference materials: CRM No. 278 (mussel tissue of *Mytilus edulis*-BCR); MA-B-3/(TM) (fish homogenate-IAEA); TORT-2 (lobster hepatopancreas-NRC-CNRC).

In 2001, samples of bottom sediments were collected at three sites (Červená Voda, Kralický Brook and Lichkov) and analysed for metal contamination. Because the river bed is covered with gravel and sand at those sites, it was only possible to collect clay sediments in a few places with a moderate flow rate. The collected sediment samples were put into polyethylene bags and stored in a freezer at  $-18^{\circ}\text{C}$ . Sediment samples were adjusted according to ISO 11466 prior to the metal contamination analysis.

The FAAS was used to analyse the sediments for Pb, Cd, Cr, Cu, Ni and Zn, and the hydride technique was used to determine the As load. The AMA 254 mercury analyser was used to determine the total mercury content in sediments.

Results were tested using ANOVA in Statgraphics software, Unistat 5.1 and MS-EXCEL 7.0. For the interpretation of the results, maximum limits for fish published in Decree No. 53/2002 of the Ministry of Health of the Czech Republic were used.

## RESULTS

In the first part of the evaluation, metal concentrations in muscle tissues of female and male fish from individual sites at the Tichá Orlice and its tributary, the Kralický Brook, from the years 2000 and 2001 were compared. Because no significant sex-related differences in metal concentrations in muscle tissues were found, all fish from individual sites ( $n = 14$ ) were used in further evaluations irrespective of their sex.

An overview of mercury and arsenic concentrations in muscle tissues of brown trout from the collection sites is in Figures 2 and 3. Cadmium, copper, zinc, chromium and nickel concentrations in muscle tissues of brown trout from collection sites at the Tichá Orlice are in Table 2. Concentrations of lead were under the detection limit of the method (0.05 mg/kg) in all samples from the years 2000 and 2001.

The highest mercury concentrations were found in brown trout caught at the Kralický Brook ( $0.37 \pm 0.08$  and  $0.40 \pm 0.08$  mg/kg) and Lichkov ( $0.41 \pm 0.10$  and  $0.34 \pm 0.07$  mg/kg) in both 2000 and 2001, and the values were very similar at both sites. The de-

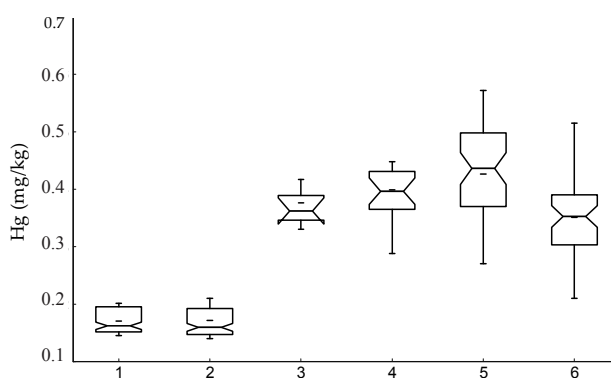


Figure 2. Comparison of mercury contents in muscles of brown trout from monitored sites of the Tichá Orlice River. 1, 2 – Červená Voda 2000, 2001; 3, 4 – Kralický 2000, 2001; 5, 6 – Lichkov 2000, 2001; bottom, top line = lower, upper quartile; middle line = median; lower, upper whisker = lower, upper adjacent value; notch: standard error

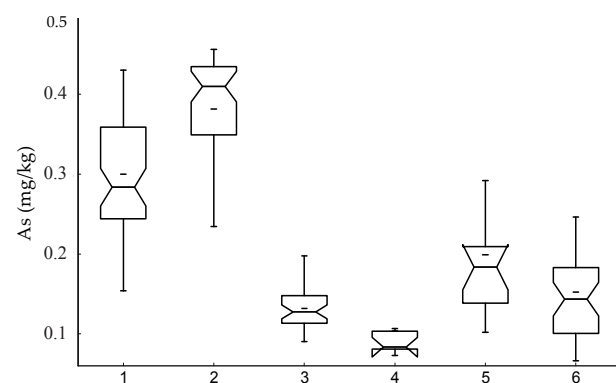


Figure 3. Comparison of arsenic contents in muscles of brown trout from monitored sites of the Tichá Orlice River. 1, 2 – Červená Voda 2000, 2001; 3, 4 – Kralický 2000, 2001; 5, 6 – Lichkov 2000, 2001; bottom, top line = lower, upper quartile; middle line = median; lower, upper whisker = lower, upper adjacent value; notch: standard error

Table 2. Content of metals in muscles of brown trout from the localities under study (2000 and 2001)

Locality	Cd	Cu	Zn	Cr	Ni
	mean $\pm$ SD (mg/kg w.w.)				
Červená Voda 2000	0.0073 $\pm$ 0.0066	0.273 $\pm$ 0.047	4.18 $\pm$ 0.98	0.081 $\pm$ 0.051	0.142 $\pm$ 0.098
Červená Voda 2001	0.0055 $\pm$ 0.0021	0.229 $\pm$ 0.051	4.25 $\pm$ 0.73	0.049 $\pm$ 0.033	0.067 $\pm$ 0.059
Králíky 2000	0.0031 $\pm$ 0.0017	0.343 $\pm$ 0.075	3.85 $\pm$ 0.94	0.072 $\pm$ 0.058	0.148 $\pm$ 0.142
Králíky 2001	0.0020 $\pm$ 0.0008	0.259 $\pm$ 0.035	4.53 $\pm$ 0.77	0.059 $\pm$ 0.040	0.102 $\pm$ 0.060
Lichkov 2000	0.0068 $\pm$ 0.0129	0.301 $\pm$ 0.084	4.31 $\pm$ 1.50	0.051 $\pm$ 0.057	0.146 $\pm$ 0.073
Lichkov 2001	0.0023 $\pm$ 0.0009	0.191 $\pm$ 0.035	3.62 $\pm$ 0.62	0.074 $\pm$ 0.044	0.083 $\pm$ 0.053

w. w. = wet weight

Table 3. Content of metals in dry matter of bottom sediments from the localities under study (2001)

Locality	Hg	As	Cd	Pb	Cu	Zn	Cr	Ni
	mg/kg d.w.							
Červená Voda	0.23	7.2	0.58	36.6	11.8	66.1	9.7	6.8
Králíky	3.69	7.7	0.78	75.5	65.2	296.7	23.2	20.1
Lichkov	0.90	6.4	0.52	31.3	20.1	83.8	13.5	9.7

d. w. = dry weight

crease in mercury concentrations in muscle tissues of the brown trout (2000 and 2001:  $0.17 \pm 0.02$  mg/kg) collected at Červená Voda (i.e. above the source of pollution) was highly significant ( $P < 0.01$ ) in both years. On the other hand, arsenic concentrations in muscle tissues of the brown trout from the same site, i.e. Červená Voda, were significantly higher ( $P < 0.01$ ) in 2000 and 2001 ( $0.29 \pm 0.08$  and  $0.37 \pm 0.07$  mg/kg) compared with arsenic concentrations found at Králíky ( $0.13 \pm 0.03$  and  $0.01 \pm 0.04$  mg/kg) and Lichkov ( $0.18 \pm 0.10$  and  $0.14 \pm 0.08$  mg/kg). Arsenic concentrations in muscle tissues of fish from Králíky and Lichkov were comparable in the two years. There were no significant differences in the concentrations of other metals monitored in the brown trout, i.e. cadmium, lead, zinc, chromium and nickel, between the three sites in 2000 or 2001. The only exception was copper, whose muscle tissue concentrations in the brown trout caught at Králíky were higher ( $P < 0.05$ ) than those found in brown trout caught at Červená Voda in 2000 and at Lichkov in 2001.

The second part of the study was a comparison between the concentrations of metals monitored

in brown trout (Hg, As, Cd, Pb, Cu, Zn, Cr, Ni) in 2000 and 2001. The comparison failed to show any significant differences between the concentrations at individual sites found in 2000 and 2001.

Metal concentrations in aquatic sediments at the collection sites are given in Table 3. It follows from the data that the highest concentrations were in samples collected from the Kralický Brook downstream from the town of Králíky. The concentrations of mercury, copper and zinc at that side were several times higher than those found at Červená Voda and Lichkov. Arsenic concentrations at all the investigated sites were practically identical.

## DISCUSSION

To assess metal contamination levels at individual sites along the upper reaches of the Tichá Orlice River, the brown trout was used as the main indicator species for the upper reaches of rivers (Studnicka *et al.*, 1974; Svobodová *et al.*, 1982; Woodward *et al.*, 1995; Clements and Rees, 1997; Eden *et al.*, 1999; Honnen *et al.*, 2001). It follows from the comparison

of metal concentrations in muscle tissues of fish caught at individual collection sites that the source of mercury contamination in the upper reaches of the Tichá Orlice River is its right-hand tributary Kralický Brook. This result is in agreement with the data reported by Lusková *et al.* (1997), who identified the plant of Tesla Králíky Company as the source of contamination. The company ended its production of fluorescent lights in 1990. Lichkov at the Tichá Orlice about 7 km downstream from its confluence with the Kralický Brook showed almost the same level of mercury contamination as the Kralický Brook itself. In brown trout caught at Kralický Brook, higher concentrations of copper ( $P < 0.05$ ) compared with the trout from Červená Voda and Lichkov were found.

Higher concentrations of mercury and copper in muscle tissues of brown trout collected at the Kralický Brook seem to copy the higher metal concentrations in aquatic sediments. The levels of zinc, chromium, nickel, lead and cadmium were, however, higher in aquatic sediments at the Kralický Brook site than in the muscle tissue of local fish. It was mentioned earlier that the river bottom at the collection sites was mostly covered with gravel and sand. For metal concentration analyses, samples of clay sediment were collected that were, however, found in a few places at the collection sites. Bottom sediments in upper reaches of rivers are not suitable indicators of contamination levels and the values ascertained should be considered as rough approximations only. Compared with mercury and arsenic, neither zinc, chromium, nickel, lead nor cadmium are considered highly accumulative metals (Svobodová *et al.*, 1996).

Arsenic concentrations in bottom sediments at all three sites were practically the same. Although significantly higher arsenic concentrations were found in the muscle tissue of brown trout at Červená Voda, no significantly higher anthropogenic contamination than at the other sites is assumed there. The Červená Voda site is situated almost on the Tichá Orlice headwaters. The characteristics of this site are different from those at downstream sites which are subject to anthropogenic (household and industrial) pollution. Pollutants at the Červená Voda site may come from agricultural activities. The evidence of it is the levels of DDT and its metabolites and HCH that were practically identical at all three sites investigated in this study (Kolářová *et al.*, 2004). No other sources of pollution were identified in the vicinity of the site. Compared with the downstream

sites, mainly its basic characteristic, i.e. water temperature, was different at the Červená Voda site. The temperature of water in the Tichá Orlice headwaters is on average 2°C lower during the vegetation period. Higher arsenic concentrations in fish tissues at lower water temperatures were demonstrated by Chan and Huff (1997) and Svobodová *et al.* (2002). Chan and Huff (1997) believed that the rate of detoxification processes slowed down at lower water temperatures. Food uptake is reduced, which leads to the reduction in the uptake of methionine, choline and proteins generally. And compared with Kralický Brook and Lichkov, Červená Voda offers significantly poorer trophic conditions, which was demonstrated both in 2000 and 2001 by the significantly lower weights of fish of the same age ( $P < 0.01$ ) from Červená Voda than from Králíky and Lichkov (Table 1).

This leads to a reduction in the elimination of methylated compounds of arsenic from the organisms and in concert of that to an increased accumulation of arsenic in tissues. Unsatisfactory results of artificial reproduction of brown trout and grayling caught at sites along the upper reaches of the Tichá Orlice River can be explained by contamination of the river with environmental endocrine disruptors (EEDs). Kolářová *et al.* (2004) found very high concentrations of vitellogen as a biochemical marker of the EED contamination in the blood plasma of male brown trout. The cause of endocrine disruption in the fish in the upper reaches of the Tichá Orlice River may be a number of organic pollutants (PAH, PCB, HCB, etc.), and it is possible that mercury compounds may also play a role in it (Keith, 1997).

The total mercury concentration found in the muscle tissue of brown trout at Kralický Brook and at Tichá Orlice – Lichkov is markedly elevated, its levels being mostly between 0.3 and 0.5 mg/kg. Some fish caught at those sites even exceed maximum limits for predatory fish, i.e. 0.5 mg/kg muscle tissue. Concentrations of the other metals in the muscle tissue of brown trout caught at all the collection sites in 2000 and 2001 were below the effective limits (As – 1, Cd – 0.05, Pb – 0.2, Cu – 10.0, Zn – 50.0, Ni – 0.05, Cr – no limit for fish – general limit 4.0 mg/kg).

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## ABSTRACT

### Posouzení kontaminace horního toku řeky Tichá Orlice kovy

Cílem této práce bylo posoudit kontaminaci řeky Tichá Orlice kovy a přispět tak k hodnocení příčin neuspokojivých výsledků reprodukce lososovitých ryb. K indikaci kontaminace byl použit pstruh obecný (*Salmo trutta morpha fario*) a orientačně též sedimenty dna. Odběry vzorků byly provedeny v měsíci červnu 2000 a 2001 na dvou lokalitách řeky Tiché Orlice (Červená Voda – 103 ř. km; Lichkov – 93 ř. km) a na jejím přítoku, Kralickém potoce (100 ř. km). Na každé lokalitě bylo v obou sledovaných obdobích odloveno po 14 kusech pstruha obecného. Ve svalovině ryb a v sedimentech dna byl metodou AAS stanoven celkový obsah rtuti, arzenu, kadmia, olova, mědi, zinku, chromu a niklu. Výraznou antropogenní kontaminaci kovy (zejména rtuť a měď) přináší do Tiché Orlice Kralický potok. Nejvyšší obsah rtuti ve svalovině pstruhů obecných byl zjištěn v lokalitě Kralický potok ( $0,37 \pm 0,08$  a  $0,40 \pm 0,08$  mg na kg) a Lichkov ( $0,41 \pm 0,10$  a  $0,34 \pm 0,07$  mg/kg) v letech 2000 a 2001 a nejnižší obsah v lokalitě Červená Voda ( $0,017 \pm 0,02$  mg/kg v obou letech). Signifikantně vyšší obsah arzenu (2000:  $0,30 \pm 0,08$  mg/kg; 2001:  $0,38 \pm 0,07$  mg/kg)

byl zjištěn ve svalovině pstruhů obecných v lokalitě Červená Voda ve srovnání s níže položenou lokalitou (Lichkov 2000;  $0,18 \pm 0,09$  mg/kg; 2001:  $0,14 \pm 0,07$  mg/kg). Autoři se domnívají, že rozdíl byl způsoben odlišnými podmínkami (především teplotou vody). Lze předpokládat, že na neuspokojivých výsledcích reprodukce ryb z horního toku Tiché Orlice se vedle organických polutantů podílejí i sloučeniny rtuti, které jsou řazeny mezi podezřelé endokrinní disruptory.

**Klíčová slova:** přítok Labe; *Salmo trutta morpha fario*; pstruh obecný; svalovina; rtuť; arzen; AAS

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